

AMENDMENTS TO THE SPECIFICATION

On page 1, paragraph beginning on line 4:

The present invention relates to a bacterial culture medium, for use under anaerobic conditions, comprising at least one metal complex which allows the oxidative polymerization of an indoxyl derivative and a substrate containing an indoxyl derivative resulting in an insoluble colored compound. Said metal complex, in particular ammoniacal iron citrate, has a concentration of between 0.3 and 0.9 mg/ml, preferably 0.6 mg/ml. Advantageously, the culture medium according to the invention may comprise a substrate such as ~~X-Gal~~ 5-bromo-4-chloro-3-indolyl-b-D-galactoside, at a concentration of between 10 and 500 mg/l.

On page 4, paragraph beginning on line 11:

The present invention relates to a bacterial culture medium, for use under anaerobic conditions, comprising at least one metal complex which allows the oxidative polymerization of an indoxyl derivative and a substrate containing an indoxyl derivative resulting in an insoluble colored compound. Said metal complex, in particular ammoniacal iron citrate, has a concentration of between 0.3 and 0.9 mg/ml, preferably 0.6 mg/ml. The culture medium according to the invention may comprise at least one selected from ~~X-Gal, X-Phos, X-aclmn, Mag-Gal, Mag- α -Gal, and Mag-Phos,~~ 5-bromo-4-chloro-3-indolyl-b-D-galactoside, 5-bromo-4-chloro-3-indolyl-phosphate, 5-bromo-4-chloro-indolyl-N-acetyl-b-D-glucosaminide, 5-bromo-6-chloro-3-indolyl-b-D-galactopyranoside, 5-bromo-6-chloro-3-indolyl- α -D-galactopyranoside, and 5-bromo-6-

chloro-3-indolyl phosphate, preferably ~~X-Gal~~ 5-bromo-4-chloro-3-indolyl-b-D-galactoside, at a concentration of between 10 and 500 mg/l, particularly between 50 and 200 mg/l, preferably at 100 mg/ml.

On page 6, paragraph beginning on line 10:

An additional aspect of the present invention relates to a combination product comprising at least one oxidizing metal complex and at least one substrate containing an indoxyl derivative resulting in an insoluble colored compound for use simultaneously, separately or spread out over time, intended for the detection of bacteria. Said substrate may be selected from ~~X-Gal, X-Phos, X-acglmn, Mag-Gal, Mag- α -Gal, and Mag-Phos,~~ 5-bromo-4-chloro-3-indolyl-b-D-galactoside, 5-bromo-4-chloro-3-indolyl-phosphate, 5-bromo-4-chloro-indolyl-N-acetyl-b-D-glucosaminide, 5-bromo-6-chloro-3-indolyl-b-D-galactopyranoside, 5-bromo-6-chloro-3-indolyl- α -D-galactopyranoside, and 5-bromo-6-chloro-3-indolyl phosphate, preferably ~~X-Gal~~ 5-bromo-4-chloro-3-indolyl-b-D-galactoside, and said metal complex is ammoniacal iron citrate.

On page 7, paragraph beginning on line 34 and commencing on page 8:

An additional aspect of the present invention relates to the use of an oxidizing metal complex, preferably ammoniacal iron citrate, for catalyzing the oxidative polymerization of indoxyl derivatives resulting in an insoluble colored compound, in particular for improving the detection of the release of an indoxyl derivative by an enzyme from a substrate containing an indoxyl derivative, it being possible for said

substrate to be a substrate selected from ~~X-Gal~~, ~~X-Phos~~, ~~X-acglnn~~, ~~Mag-Gal~~, ~~Mag- α -Gal~~, and ~~Mag-Phos~~, 5-bromo-4-chloro-3-indolyl-b-D-galactoside, 5-bromo-4-chloro-3-indolyl-phosphate, 5-bromo-4-chloro-indolyl-N-acetyl-b-D-glucosaminide, 5-bromo-6-chloro-3-indolyl-b-D-galactopyranoside, 5-bromo-6-chloro-3-indolyl- α -D-galactopyranoside, and 5-bromo-6-chloro-3-indolyl phosphate, preferably ~~X-Gal~~5-bromo-4-chloro-3-indolyl-b-D-galactoside. Said metal complex makes it possible to intensify the colored halo and/or to increase the color of the colonies. Indeed, it reacts with the indoxyl derivative according to the invention to give a colored compound which precipitates.

On page 9, paragraph beginning on line 5:

A strain of *Bifidobacterium bifidum* was inoculated into the cysteinated Columbia medium + ~~X-Gal~~ 5-bromo-4-chloro-3-indolyl-b-D-galactoside with or without AIC (0.3 g/l), and then the effect of the addition of AIC was tested before or after autoclaving/regeneration.

On page 10, paragraph beginning on line 10:

The presence of AIC made it possible to observe the color due to the hydrolysis of the ~~X-Gal~~ 5-bromo-4-chloro-3-indolyl-b-D-galactoside substrate for the *Clostridium* colonies:

On page 10, paragraph beginning on line 37 and commencing on page 11:

The colonies of *C. butyricum* (β -galactosidase+) are cream-colored without AIC; pink with a pink halo with AIC, and those of *Citrobacter* (β -galactosidase+) are cream-

colored without AIC; pink with AIC. The presence of AIC made it possible to observe the coloring due to the hydrolysis of the ~~Mag-Gal~~ 5-bromo-6-chloro-3-indolyl-b-D-galactopyranoside substrate for the colonies of *Clostridium butyricum* and for *Citrobacter*. The enzymes of the latter would therefore not be inducible but would be more capable of hydrolzing ~~Mag-Gal~~ 5-bromo-6-chloro-3-indolyl-b-D-galactopyranoside than ~~X-Gal~~ 5-bromo-4-chloro-3-indolyl-b-D-galactoside. The differences between the medium with AIC and the medium without AIC are, in order, greatly marked for the strain of: *C. perfringens*, *C. butyricum*, *Citrobacter*.

On page 12, paragraph beginning on line 17:

The differences between the medium with AIC and the medium without AIC are very marked for the strain of: *Citrobacter*, then there are in order: *C. butyricum*, *C. perfringens*, *E. coli* and finally *B. fragilis*. Even if the colors are not sharp, the presence of AIC made it possible to observe the color (*C. perfringens*, *C. butyricum*), or to increase the color (*E. coli*) due to the hydrolysis of the ~~X-Phos~~ 5-bromo-4-chloro-3-indolyl-phosphate substrate. As regards *B. fragilis*, this releases the extra cellular enzymes which form halos of undefinable colors.

On page 13, paragraph beginning on line 5:

The presence of AIC made it possible to observe the color due to the hydrolysis of the ~~Mag-Phos~~ 5-bromo-6-chloro-3-indolyl phosphate substrate for the colonies of *Clostridium*, *E. coli* and *Citrobacter*.

On page 13, paragraph beginning on line 16:

The colonies of *C. perfringens* (~~Mag- α -Gal~~ 5-bromo-6-chloro-3-indolyl- α -D-galactopyranoside+) are cream-colored without AIC; pink in the presence of AIC. Those of *Bacteroides fragilis* (~~Mag- α -Gal~~ 5-bromo-6-chloro-3-indolyl- α -D-galactopyranoside+) are dark cream-colored without AIC; cream-pink with AIC. Those of *C. butyricum* (~~Mag- α -Gal~~ 5-bromo-6-chloro-3-indolyl- α -D-galactoside +), *Citrobacter* and *E. coli* are cream-colored without AIC; darker or light cream-colored with AIC.

The differences between the medium with AIC and the medium without AIC are more marked for the strains of: *C. perfringens* and *B. fragilis*, then there are *C. butyricum*, *Citrobacter* and *E. coli*.

On page 13, paragraph beginning on line 32 and commencing on page 14:

The colonies of *C. perfringens* (~~X- α -glu~~ 5-bromo-4-chloro-indolyl-N-acetyl-b-D-glucosaminide+) are cream-colored without AIC; very slightly greenish cream-colored in the presence of AIC. Those of *C. butyricum* (~~X- α -glu~~ 5-bromo-4-chloro-indolyl-N-acetyl-b-D-glucosaminide-) do not exhibit growth without AIC; are greenish with a blue halo in the presence of AIC. *Bacteroides fragilis* (~~X- α -glu~~ 5-bromo-4-chloro-indolyl-N-acetyl-b-D-glucosaminide+), *Citrobacter* and *E. coli* are cream-colored with or without AIC.

On page 14, paragraph beginning on line 22:

The colonies of *Clostridium perfringens* are black. Whether ~~X-Gal~~ 5-bromo-4-chloro-3-indolyl-b-D-galactoside, ~~Mag-Phos~~ 5-bromo-6-chloro-3-indolyl phosphate, X-

glu or X-glucu is added, the colors due to the hydrolysis of these substrates remain difficult to see. Nevertheless, the blue-gray halos around the colonies of *C. perfringens* in the presence of ~~X-Gal~~ 5-bromo-4-chloro-3-indolyl-b-D-galactoside (100 mg/l, combined with ~~Mag-Phos~~ 5-bromo-6-chloro-3-indolyl phosphate 50 m/l or with X-glucu 100 m/l) can make it possible to distinguish between the *C. perfringens* and the other microorganisms. These halos are also observed around colonies of *E. coli* which are all blue (solely as ~~X-Gal~~ 5-bromo-4-chloro-3-indolyl-b-D-galactoside + ~~Mag-Phos~~ 5-bromo-6-chloro-3-indolyl phosphate).

On page 15, paragraph beginning on line 4:

The substrates ~~X-Gal, Mag-Gal, X-Phos~~ 5-bromo-4-chloro-3-indolyl-b-D-galactoside, 5-bromo-6-chloro-3-indolyl-b-D-galatopyranoside, 5-bromo-4-chloro-3-indolyl-phosphate and ~~Mag-Phos~~ 5-bromo-6-chloro-3-indolyl-phosphate (100 mg/l) were tested in the presence and in the absence of AIC (0.6 g/l) by reusing the base of the TSC medium (TSC medium without antibiotic, and this time without disulfite). The colors are in general less sharp than in Columbia medium.

On page 15, paragraph beginning on line 13:

With ~~X-Gal~~ 5-bromo-4-chloro-3-indolyl-b-D-galactoside, the differences between the medium with AIC and the medium without AIC are more marked for the strains of: *C. perfringens* and *E. coli*, and then *Citrobacter* and *B. fragilis*. *C. butyricum* remains cream-colored with AIC. With Mag-Gal, the differences between the medium with AIC and the medium without AIC are more marked for the strain of: *C. perfringens* and then for

those of *E. coli* and *Citrobacter*, and finally *B. fragilis*. *C. butyricum* did not grow in the absence of AIC.

On page 15, paragraph beginning on line 31:

With ~~Mag-Phos~~5-bromo-6-chloro-3-indolyl, the colonies are just darker in the presence of AIC, the differences between the medium with AIC and the medium without AIC are more marked than the strains of *E. coli* and *Citrobacter* and then for that of *C. perfringens*.

On page 17, table II:

Table II

	<i>C.perfringens</i>	<i>C.butyricum</i>	<i>Citrobacter</i>	<i>E.coli</i>	<i>B.fragilis</i>
X-Gal <u>5-bromo-4-chloro-3-indolyl-b-D-galactoside</u>	3	2	0	Ø	Ø
X-Gal <u>5-bromo-4-chloro-3-indolyl-b-D-galactoside</u> TSC	3	0	2	3	2
Mag-Gal <u>5-bromo-6-chloro-3-indolyl-b-D-galactopyranoside</u>	3	2	1	Ø	Ø
Mag-Gal <u>5-bromo-6-chloro-3-indolyl-b-D-galactopyranoside</u> de TSC	3	Ø	2	2	1
GALACT-OSIDASE	3	133	125	25	15
X-Phos <u>5-</u>	2	2	3	2	1

<u>bromo-4-chloro-3-indolyl-phosphate</u>					
X-Phos 5-bromo-4-chloro-3-indolyl-phosphate TSC	2	Ø	3	3	2
Mag-phos 5-bromo-6-chloro-3-indolyl-phosphate	3	3	2	2	1
Mag-phos 5-bromo-6-chloro-3-indolyl-phosphate TSC	2	0	3	3	0
PHOSPHATASE	225	166	275	25	1

On page 18, paragraph beginning on line 10:

Ferricyanide was used alone at 0.6 g/l. There is no difference between the media containing these products or otherwise for the substrate ~~X-Gal~~ 5-bromo-4-chloro-3-indolyl-b-D-galactoside. Only the colonies of *Bacteroides fragilis* are blue with ~~X-Phos~~ 5-bromo-4-chloro-3-indolyl-phosphate in the presence of ferricyanide. Thus, ~~X-phos~~ 5-bromo-4-chloro-3-indolyl-phosphate + ferricyanide may be an excellent medium for preidentifying *Bacteroides*.

On page 19, paragraph beginning on line 4:

The following two strains of *Clostridium* grow:

- the colonies of *C. butyricum* remain cream-colored,
- those of *C. perfringens* are slightly colored (colonies having a pink center with ~~Mag~~ Gal-5-bromo-6-chloro-3-indolyl-b-D-galactopyranoside (100 mg/l) and AIC (0.6 g/l) and colonies having a greenish center surrounded by a very slight halo with ~~Gal-5-bromo-4-chloro-3-indolyl-b-D-galactoside~~ (100 mg/l) and AIC (0.6 g/l)).